# DERIVATISED HYDROGELS AND THEIR USE

This invention relates to polymeric materials and particularly, although not exclusively, relates to such materials in the form of hydrogels. Preferred embodiments relate to the use of a polymeric material in a biological application, such as in tissue engineering, for example to support cell amplification; as a vehicle for cell or tissue grafting or transplantation; and/or as a basis for "smart" wound dressings.

Several types of traumatic injury and several disease states can affect quality of life by prejudicing normal functioning of different organ systems. Examples include lesions to the cornea of the eye, impact and abrasive damage to the surface of articular cartilage, and acute trauma or chronic wounds affecting the skin. In the absence of sufficient or appropriate intact tissues for transplantation or grafting to treat such conditions, one potential therapeutic strategy would be to take a small biopsy from the patient and increase the numbers of cells in vitro using culture techniques to produce autologous tissues for use in treating the same patient. Certain cells when grown in culture will in any case re-establish simple tissue architectures such as epithelium given appropriate conditions. Cell cultures can be harvested and deployed from such tissue engineered epithelial sheets in several different ways. For example, the cells may be transferred to another surface to which they adhere and can continue to survive. One object of the invention is to provide a polymeric material that can support cell growth/amplification.

So called "smart" dressings are known which are adapted to facilitate the two cellular processes which are pivotal to successful wound repair, namely cell division and cell migration. Both of the processes are substratum dependent. Pathological conditions that impair or impede either of the processes can lead to the formation of chronic, poorly healing wounds and possibly significant scarring. Another object of the present invention is to provide a polymeric material that can be used in a "smart" dressing.

Polymeric materials in the form of hydrogels are known for a range of uses. WO98/12239 (University of Bradford) describes a range of such hydrogels. Hydrogels comprising polyvinylalcohol cross-linked by glutaraldelyde are also known. However, such known hydrogels are not in themselves generally suitable for use in cell amplification or smart dressings. It is one object of the present invention to provide a hydrogel which has a wide range of useful applications.

According to a first aspect of the invention there is provided a method of derivatising a polymeric material of a type which includes encapsulated water, the method comprising:

(a) selecting a first hydrated polymeric material which includes encapsulated water;
(b) reducing the level of encapsulated water in said first hydrated polymeric material to produce a second polymeric material;

(c) treating said second polymeric material with derivatisation means for derivatising said second polymeric material.

Said first hydrated polymeric material is preferably a hydrogel. A said hydrogel may be defined as a cross-linked, water insoluble, water containing material.

Said first polymeric material or hydrogel suitably contains at least 40wt%, preferably at least 55wt%, more preferably at least 70wt%, especially at least 80wt% water. The amount of water may be less than 95wt%, preferably less than 90wt%. The level of water may be determined by any suitable means, for example by thermogravimetric analysis.

The difference between the wt% of water in said first polymeric material and that in said second polymeric material may be at least 40wt%, preferably at least 55wt%, more preferably at least 70wt%. The ratio of the amount (wt%) of water in the first polymeric material to that in said second polymeric material may be at least 10, suitably at least 20, preferably at least 30, more preferably at least 40, especially at least 50. The ratio is preferably less than 1000.

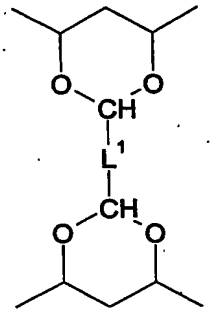
Said second polymeric material suitably includes less than 10wt%, preferably less than 5wt%, more preferably less than 2wt%, especially less than 1wt% of encapsulated water. Said second polymeric material may include a trace, for example at least 0.01wt% of encapsulated water.

Said first hydrated polymeric material preferably comprises a third polymeric material which is cross-linked by a cross-linking means. Said first polymeric material may be prepared by selecting a third polymeric material and treating it with a said cross-linking means. Wherein said third polymeric material may include functional groups selected from hydroxy, carboxylic acid, carboxylic acid derivatives (e.g. ester) and amine groups. Said third polymeric material preferably includes a backbone comprising, preferably consisting essentially of carbon atoms. The backbone is preferably saturated. Pendent from the backbone are one or more said functional groups described. Said third polymeric material may have a molecular weight of at least 10,000. Said third polymeric material is preferably a polyvinyl polymer. Preferred third polymeric materials include optionally substituted, preferably unsubstituted, polyvinylalcohol, polyvinylacetate, polyalkylene glycols, for example polypropylene glycol, and collagen (and any component thereof). Polyvinylalcohol is an especially preferred third polymeric material.

In especially preferred embodiments said first polymeric material include cross-linked polyvinyl alcohol.

A preferred cross-linking means comprises a chemical cross-linking material. Such a material is preferably a polyfunctional compound having at least two functional groups capable of reacting with functional groups of said third polymeric material. Preferably, said cross-linking material includes one or more of carbonyl, carboxyl, hydroxy, epoxy, halogen or amino functional groups which are capable of reacting with groups present along the

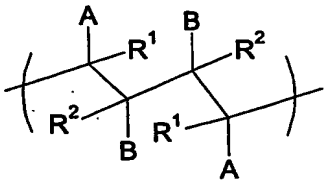
polymer backbone or in the polymer structure of the third polymeric material. Preferred cross-linking materials include at least two aldehyde groups. Thus, in a preferred embodiment, said first polymeric material includes a material formed by cross-linking polyvinylalcohol using a material having at least two aldehyde groups. Thus, said first polymeric material preferably includes a moiety of formula I.



I

wherein $L^1$ is a residue of said cross-linking material.

Said cross-linking material preferably comprises a fourth polymeric material. Said fourth polymeric material preferably includes a repeat unit of formula



II

wherein A and B are the same or different, are selected from optionally-substituted aromatic and heteroaromatic

groups and at least one comprises a relatively polar atom or group and $R^1$ and $R^2$ independently comprise relatively non-polar atoms or groups.

A and/or B could be multi-cyclic aromatic or heteroaromatic groups. Preferably, A and B are independently selected from optionally-substituted five or more preferably six-membered aromatic and heteroaromatic groups. Preferred heteroatoms of said heteroaromatic groups include nitrogen, oxygen and sulphur atoms of which oxygen and especially nitrogen, are preferred. Preferred heteroaromatic groups include only one heteroatom. Preferably, a or said heteroatom is positioned furthest away from the position of attachment of the heteroaromatic group to the polymer backbone. For example, where the heteroaromatic group comprises a six-membered ring, the heteroatom is preferably provided at the 4-position relative to the position of the bond of the ring with the polymeric backbone.

Preferably, A and B represent different groups. Preferably, one of A or B represents an optionally-substituted aromatic group and the other one represents an optionally-substituted heteroaromatic group. Preferably A represents an optionally-substituted aromatic group and B represents an optionally-substituted heteroaromatic group especially one including a nitrogen heteroatom such as a pyridinyl group.

Unless otherwise stated, optionally-substituted groups described herein, for example groups A and B, may be substituted by halogen atoms, and optionally substituted alkyl, acyl, acetal, hemiacetal, acetalalkyloxy, hemiacetalalkyloxy, nitro, cyano, alkoxy, hydroxy, amino,

alkylamino, sulphinyl, alkylsulphinyl, sulphonyl, alkylsulphonyl, sulphonate, amido, alkylamido, alkylcarbonyl, alkoxycarbonyl, halocarbonyl and haloalkyl groups. Preferably, up to 3, more preferably up to 1 optional substituents may be provided on an optionally substituted group.

Unless otherwise stated, an alkyl group may have up to 10, preferably up to 6, more preferably up to 4 carbon atoms, with methyl and ethyl groups being especially preferred.

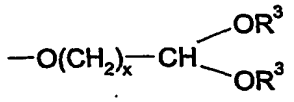
Preferably, A and B each represent polar atoms or group -that is, there is preferably some charge separation in groups A and B and/or groups A and B do not include carbon and hydrogen atoms only.

Preferably, at least one of A or B includes a functional group which can undergo a condensation reaction, for example on reaction with said third polymeric material. Preferably, A includes a said functional group which can undergo a condensation reaction.

Preferably, one of groups A and B includes an optional substituent which includes a carbonyl or acetal group with a formyl group being especially preferred. The other one of groups A and B may include an optional substituent which is an alkyl group, with an optionally substituted, preferably unsubstituted, $C_{1-4}$ alkyl group, for example a methyl group, being especially preferred.

Preferably, A represents a group, for example an aromatic group, especially a phenyl group, substituted (preferably at the 4-position relative to polymeric backbone when A

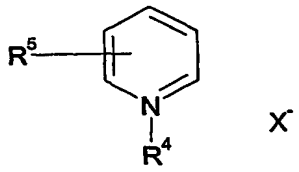
represents an optionally-substituted phenyl group) by a formyl group or a group of general formula



III

where x is an integer from 1 to 6 and each $R^3$ is independently an alkyl or phenyl group or together form an alkalene group.

Preferably, B represents an optionally-substituted heteroaromatic group, especially a nitrogen-containing heteraromatic group, substituted on the heteroatom with a hydrogen atom or an alkyl or aralkyl group. More preferably, B represents a group of general formula



IV

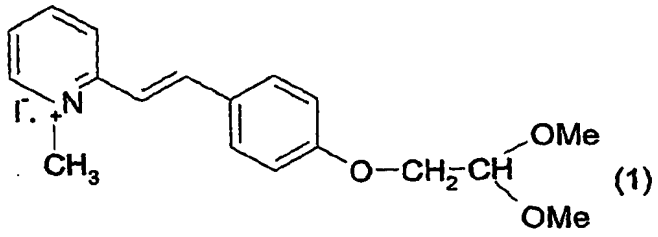
wherein $R^4$ represents a hydrogen atom or an alkyl or aralkyl group, $R^5$ represents a hydrogen atom or an alkyl group and $X^-$ represents a strongly acidic ion.

Preferably, $R^1$ and $R^2$ are independently selected from a hydrogen atom or an optionally-substituted, preferably unsubstituted, alkyl group. Preferably, $R^1$ and $R^2$ represent the same atom or group. Preferably, $R^1$ and $R^2$ represent a hydrogen atom.

Preferred fourth polymeric materials may be prepared from any of the following monomers by the method described in WO98/12239 and the content of the aforementioned document is incorporated herein by reference:

α-(p-formylstyryl)-pyridinium, γ-(p-formylstyryl)-pyridinium, α-(m-formylstyryl)-pyridinium, N-methyl-α-(p-formylstyryl)-pyridinium, N-methyl-β-(p-formylstyryl)-pyridinium, N-methyl-α-(m-formylstyryl)-pyridinium, N-methyl-α-(o-formylstyryl)-pyridinium, N-ethyl-α-(p-formylstyryl)-pyridinium, N-(2-hydroxyethyl)-α-(p-formylstyryl)-pyridinium, N-(2-hydroxyethyl)-γ-(p-formylstyryl)-pyridinium, N-allyl-α-(p-formylstyryl)-pyridinium, N-methyl-γ-(p-formylstyryl)-pyridinium, N-methyl-γ-(m-formylstyryl)-pyridinium, N-benzyl-α-(p-formylstyryl)-pyridinium, N-benzyl-γ-(p-formylstyryl)-pyridinium and N-carbamoylmethyl-γ-(p-formylstyryl)-pyridinium. These quaternary salts may be used in the form of hydrochlorides, hydrobromides, hydroiodides, perchlorates, tetrafluoroborates, methosulfates, phosphates, sulfates, methane-sulfonates and p-toluene-sulfonates.

Also, the monomer compounds may be styrylpyridinium salts possessing an acetal group, including the following: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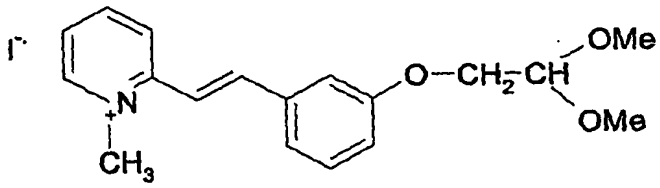
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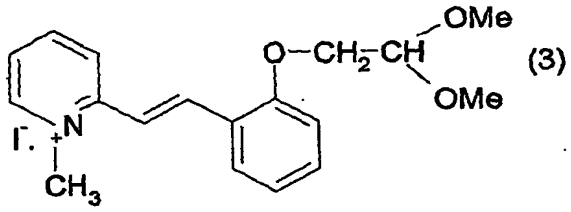
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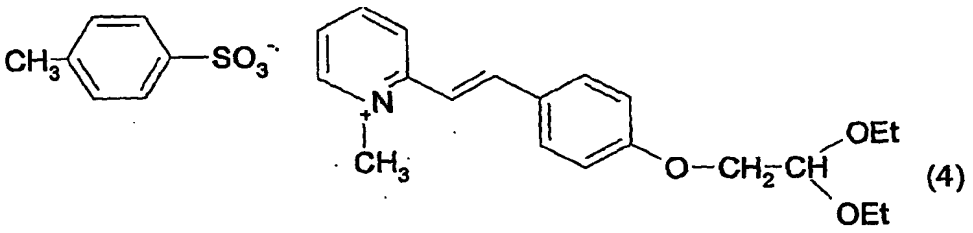
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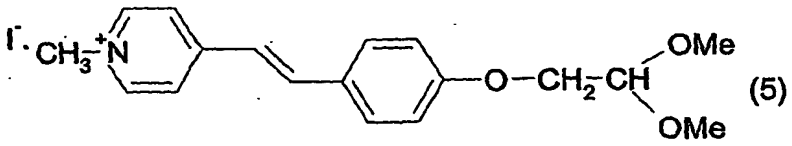
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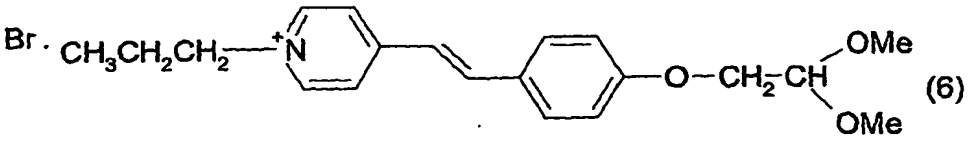
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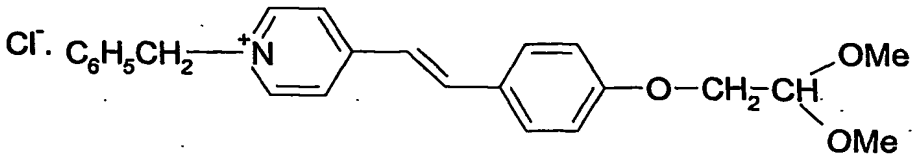
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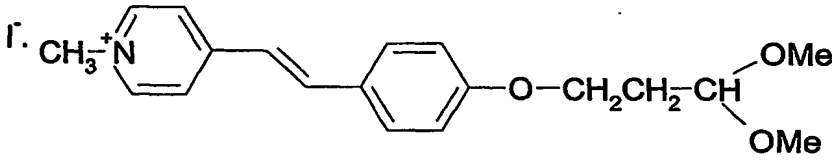
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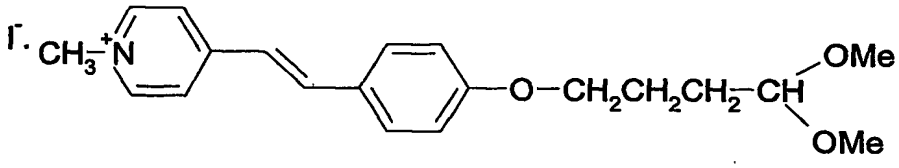
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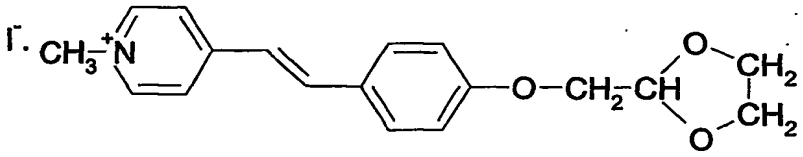
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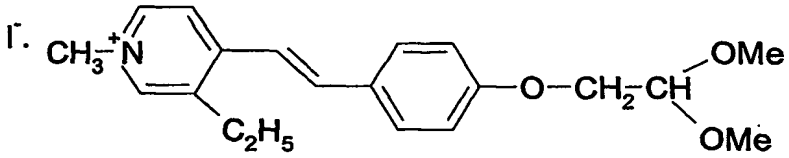
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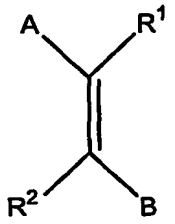
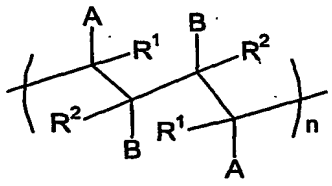
Thus, said fourth polymeric material is preferably prepared or preparable by providing a compound of general formula




wherein A, B, $R^1$ and $R^2$ are as described above, in an aqueous solvent, (suitably so that molecules of said monomer aggregate) and causing the groups C=C in said compound to react with one another, (for example using UV radiation,) to form said fourth polymeric material.

Said fourth polymeric material may be of formula




V

wherein A, B, $R^1$ and $R^2$ are as described above and n is an integer. Integer n is suitably 10 or less, preferably 8 or less, more preferably 6 or less, especially 5 or less. Integer n is suitably at least 1, preferably at least 2, more preferably at least 3. Preferably, formation of said first polymeric material from said third and fourth polymeric materials involves a condensation reaction. Preferably, formation of said first polymeric material involves an acid catalysed reaction. Preferably, said third and fourth polymeric materials include functional groups which are arranged to react, for example to undergo

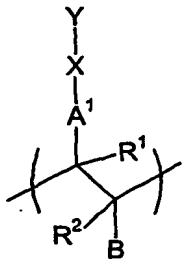
a condensation reaction, thereby to form said first polymeric material. Preferably, said third and fourth polymeric materials include functional groups which are arranged to react for example to undergo an acid catalysted reaction thereby to form said first polymeric material.

Said first polymeric material may be prepared by providing a mixture of said third polymeric material and said cross-linking material, especially said fourth polymeric material described, and causing the two materials to react. Preferably, said mixture includes at least 2wt%, more preferably at least 3wt% of said third polymeric material. When the molecular weight of the third polymeric material is relatively low (e.g. 50,000) the maximum amount of said third polymeric material in the mixture may be up to 40wt%. When the molecular weight of the third polymeric material is higher then the maximum amount may be less, for example up to 30wt%, or up to 20wt%. Said mixture may include at least 0.05wt%, preferably at least 0.1 wt% of said cross-linking means, especially said fourth polymeric material. The amount of said cross-linking means may be up to 3wt%.

Said third polymeric material and said cross-linking means are preferably provided in water. Said mixture may include at least 80wt%, suitably includes at least 85wt%, preferably includes at least 92wt%, more preferably includes at least 95wt%, especially includes at least 96wt% water. Said mixture may include other minor components, for example a catalyst, especially an acid, for catalysing the formation of said first polymeric

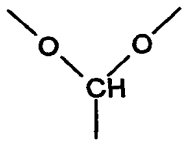
material from said third polymeric material and said cross-linking means.

Said first polymeric material suitably includes a moiety of formula



VI

wherein $R^1$, $R^2$ and B are as described above, $A^1$ represents a residue of group A described above after the reaction involving said third and fourth polymeric materials, Y represents a residue of said third polymeric material after said reaction involving said third and fourth polymeric materials and X represents a linking atom or group extending between the residues of said third and fourth polymeric materials. In one preferred embodiment $A^1$ represents an optionally-substituted phenyl group, X represents a group



which is bonded via the oxygen atoms to a residue of said third polymeric material. For example, group X may be

bonded to the polymer backbone of said third polymeric material.

The level of water may be reduced by any suitable means in step (b). Suitably, drying is undertaken at a temperature within the range 10°C to 60°C under atmospheric pressure or a lower pressure such as in a vacuum.

In step (c), the second polymeric containing a relatively low level of encapsulated water is treated with said derivatisation means. Derivatisation of said second polymeric material preferably includes a series of steps. In a first derivatisation step, said second polymeric material may be treated with a first derivatisation material which suitably reacts with said second polymeric material. A complex may be formed between the second polymeric material and said first derivatisation material or, preferably, reaction involves the formation of covalent bonds between the second polymeric material and the first derivatisation material. Preferably, the reaction of said second polymeric material and said first derivatisation material is carried out in the presence of less than 5wt%, preferably less than 1 wt% water. Preferably, the reaction is carried out in an organic solvent (e.g. acetone). Preferably, the reaction is carried out substantially in the absence of water. In this case, the reaction may predominantly take place on the surface of the second polymeric material with little penetration and reaction of reactants in microvoids in the polymeric material from which microvoids encapsulated water has been removed in step (b) of the method.

Said first derivatisation material may have any feature of the chemical cross-linking materials referred to above. When the first derivatisation step is carried out in an organic solvent and substantially in the absence of water, such a first derivatisation material should not (in the first derivatisation step) cross-link parts of the second polymeric material to any significant degree. Said first derivatisation material is preferably di-functional and preferably only one functional group of each molecule of the material reacts with the second polymeric material in said first derivatisation step. Preferably, said first derivatisation material includes at least one aldehyde group.

Said first derivatisation material may be selected from the monomers described above from which said fourth polymeric material may be prepared.

Derivatisation of the second polymeric material may include one or more derivatisation steps (including said first step described) arranged to introduce a linking moiety on said second polymeric material. The linking moiety is suitably arranged to link the second polymeric material to an active moiety. An active moiety may be selected to have desired properties and thereby provide a means whereby the desired properties may be associated with the derivatised material produced in the method. For example, the active material may be bio-compatible (and may therefore be arranged to increase the bio-compatibility of the first polymeric material) and/or it may be arranged to increase adhesion to cells. Preferred active materials include amino acid containing moieties, peptides and proteins. Alternatively, an active moiety

may comprise a conducting polymer, organic semi-conductor or another material relevant to microelectronics interfacing technologies. In this case, the active moiety may be part of a sensor for monitoring cell chemistry or biology.

In some situations it may be possible to introduce an active moiety of the type described as part of said first step. However, said active moiety is suitably introduced in a step subsequent to said first step.

As described above, said first derivatisation step is suitably carried out substantially in the absence of water. One or more subsequent steps may also be carried out substantially in the absence of water. Alternatively, a subsequent derivatisation step may be carried out in the presence of water. Since it may be preferred that reactants in such a subsequent derivatisation step do not substantially penetrate into microvoids and react with functional groups other than those produced by derivatisation of said second polymeric material by said derivatisation means, when a derivatisation step is carried out in the presence of water, reactants and/or conditions are selected so the derivatisation step involves reaction with functional groups formed in an earlier derivatisation step. To achieve this, derivatisation may involve use of a material having a functional group which is unable to react with functional groups of the polymeric material other than those formed in an earlier derivatisation step or the speed of reaction of a selected material with groups formed in an earlier derivatisation step may be quicker than for other functional groups of the particular polymeric material.

Derivatisation of the second polymeric material preferably include a derivatisation step in which a compound having a amine group is reacted with the second polymeric material or a derivative thereof, for example a derivative which includes a linking group as described above. Derivatisation with an amine group containing compound is preferably carried out in an aqueous solvent.

The method of the first aspect may involve increasing the level of encapsulated water after step (b). The level may be increased during treatment with derivatisation means in step (c) or subsequently. Advantageously, the strength of the first polymeric material after derivatisation and rehydration as described is comparable (in some cases it may be higher in some respects) to that of the first polymeric material selected in step (a).

The first polymeric material selected in step (a) may include predetermined microtopography and/or surface patterning and/or shape. Advantageously, in carrying out the method described, the microtopography, surface patterning and/or shape may be substantially retained after derivatisation of the material.

According to a second aspect of the invention, there is provided a method of making a polymeric material, the method comprising:

(a) selecting a fifth polymeric material which comprises:

(i) a third polymeric material as described according to said first aspect cross-linked by a

fourth polymeric as described according to said first aspect; or

(ii) a polymeric material which includes a moiety of formula VI wherein $R^1$, $R^2$, B, $A^1$, X and Y are as described according to said first aspect; and

(b) treating said fifth polymeric material with derivatisation means for derivatising said fifth polymeric material, said derivatisation means being as described according to said first aspect.

Any feature of the first aspect may be applied to the second aspect mutatis mutandis.

The method of the first and second aspects may be used to introduce micropatterned surface chemistry on surfaces of a polymeric material derivatised in the methods. In this regard polymeric materials may be derivatised with a first moiety at predetermined positions on their surfaces with other positions on the surfaces being underivatised or dervatised with a different second moiety. In one embodiment, the first moiety may be arranged to render positions of the surface highly bio-compatible (e.g. adhesive of cells) whereas remaining areas of the surface may be less bio-compatible and/or may be arranged to block cell attachment.

Micropatterned surface chemistry may be produced by contacting the surface of the polymeric material at predetermined positions with a derivatisation means, for example a compound have an amine group as described above.

According to a third aspect of the invention, there is provided a derivatised polymeric material prepared or

preparable in a method according to said first aspect or said second aspect.

According to a fourth aspect of the invention, there is provided a derivatised hydrogel. The hydrogel may be as described according to the first or second aspects.

Said hydrogel of the fourth aspect is preferably predominantly derivatised on surface regions thereof, in preference to macrovoids of the gel.

The derivatised polymeric material or hydrogel of the third and fourth aspects may include amide groups. Said amide groups are preferably part of a moiety pendent from a polymeric backbone. The amide groups preferably link a derivatisation means to the polymeric backbone. The amide groups preferably link an active material, for example an amino acid containing material, to a part of a moiety pendent from said polymeric backbone.

According to a fifth aspect of the invention, there is provided a method of preparing a material for a biological application, the method comprising forming microtopographical features in a surface of a first or second polymeric material according to said first aspect; or a fifth polymeric material according to said second aspect.

Said microtopographical features are preferably predetermined.

The polymeric material treated according to the third aspect may encapsulate water prior to, during and/or after

formation of said microtopographical features. The method may include a step of reducing the level of encapsulated water after microtopographical features have been formed and optionally derivatising the polymeric material which includes said microtopographical features as described according to step (c) of the first aspect and step (b) of the second aspect. Advantageously, derivatisation may be restricted to predetermined positions on the surface of the polymeric material thereby to introduce micropatterned surface chemistry. The material produced may therefore incorporate both three dimensional surface patterning and patterned surface chemistry which may have benefits in certain biological applications such as dressings for wounds (or the like).

The formation of microtopographical features as described according to the fifth aspect may involve forming a template, for example a grating slide, incorporating a desired topography and contacting the template with a polymeric material in which the microtopographic features are to be formed. Preferably, contact with the polymeric material takes place prior to complete polymerisation of the polymeric material and contact continues as the material polymerises. When a polymeric material in which microtopographic features are to be formed is made from said third and fourth polymeric materials described above, the method may involve: mixing the third and fourth polymeric materials with any catalyst required, contacting the mixture with the template, and effecting reaction of the third and fourth polymeric materials; or a mixture comprising the third and fourth polymeric materials may be at least partially reacted prior to contact with the

template. Thereafter, the reaction of the third and fourth polymeric materials may be completed.

Said microtopographical features may be predetermined by the provision of a template which is used to define the features in the method. The template may represent a negative of features produced in the first polymeric materials treated in the method of the fifth aspect.

Microtopographical features formed in accordance with said fifth aspect may comprise uniform shapes in the surface, for example channels or grooves which may have dimensions of less than 100$\mu$m.

According to a sixth aspect of the invention, there is provided a polymeric material, preferably for a biological application, the material comprising a polymeric material or hydrogel as described herein having microtopographical features.

The polymeric material or hydrogel may be as described according to the third or fourth aspects. Thus preferably, the invention provides a hydrogel having microtopographical features wherein said hydrogel is derivatised and preferably is derivated by an amino acid containing material.

According to a seventh aspect of the invention, there is provided a wound care product comprising a derivatised polymeric material or hydrogel according to any of the third, fourth, fifth or sixth aspects.

The wound care product may be a plaster or bandage (or the like) or, when usable internally, could be an implantable prosthesis (or the like). The term "wound" is intended to encompass defects caused by damage and/or disease.

According to an eight aspect of the invention, there is provided a method of treatment of the human or animal body, for example the treatment of damaged and/or diseased tissue and/or wounds, the method comprising positioning a derivatised polymeric material, hydrogel or wound care product according to the third, fourth, fifth, sixth or seventh aspects on or adjacent an area to be treated.

According to a ninth aspect of the present invention, there is provided the use of a polymeric material or hydrogel according to the third, fourth, fifth or sixth aspects for the manufacture of a material for treatment of damaged and/or diseased tissues and/or wounds.

Any feature of any aspect of any invention or embodiment described herein may be combined with any feature of any aspect of any other invention or embodiment described herein.

Specific embodiments of the invention will now be described, by way of example, with reference to Figure 1 which is a schematic representation of the derivatisation of a hydrogel with fibronectin.

The following examples describe how a hydrogel may be prepared and manipulated for use as a "smart" dressing. An objective in the preparation of a smart dressing is to recreate using microengineering techniques the

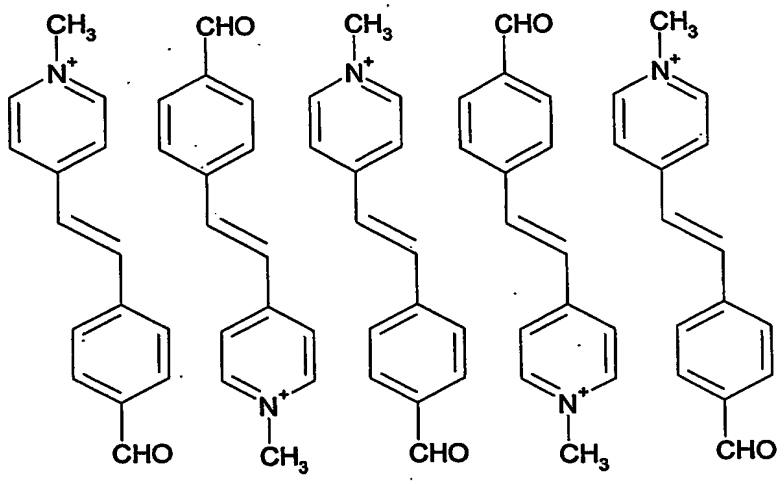
microenvironmental conditions of the remodelling extracellular matrix of various types of wound which can then serve as an alternative highly permissive substratum for cell migration and cell division. To achieve oriented cell migration and division, it is important to present adherent cells with oriented surface shape in the form of microtopograhic guidance cues or micropatterned surface chemistry, in particular that required to initiate and accelerate cell motility. In general terms, in the embodiments which follow, means are provided whereby the microtopography of a surface of a hydrogel can be manipulated in a predetermined manner and, thereafter, the chemistry at the surface can be adjusted thereby to provide a material which can be used to achieve oriented cell migration and division.

Example 1 - General method of preparing hydrogel

Step (a) - Preparation of poly (1,4-di(4-(N-methylpyridinyl))-2,3-di(4-(1-formylphenyl)butylidene

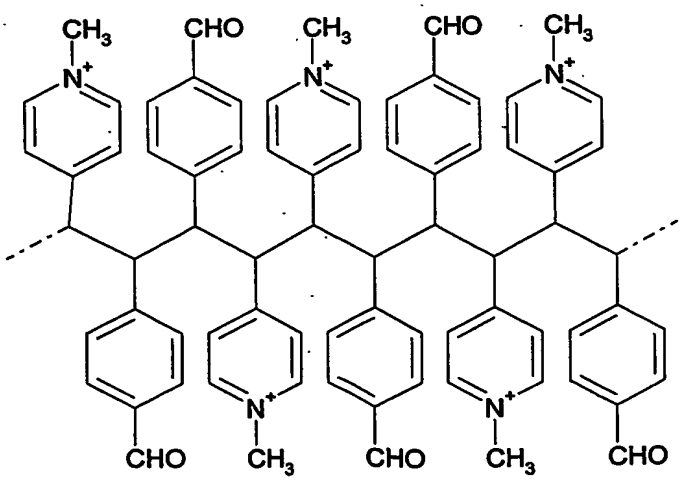
This was prepared as described in Example 1 of PCT/GB97/02529, the contents of which are incorporated herein by reference. In the method, an aqueous solution of greater than 1 wt% of 4-(4-formylphenylethenyl)-1-methylpyridinium methosulphonate (SbQ) is prepared by mixing the SbQ with water at ambient temperature. Under such conditions, the SbQ molecules form aggregates. The solution was then exposed to ultraviolet light. This results in a photochemical reaction between the carbon-carbon double bonds of adjacent 4-(4-formylphenylethenyl)-1-methylpyridinium methosulphate molecules (VIII) in the aggregate, producing a polymer, poly (1,4-di(4-(N-

methylpyridinyl))-2,3-di(4-(1-formylphenyl)butylidene methosulphonate (IX), as shown in the reaction scheme below. It should be appreciated that the anions of compounds VIII and IX have been omitted in the interests of clarity.



VIII

>1%w/w Aqueous solution
UV irradiation



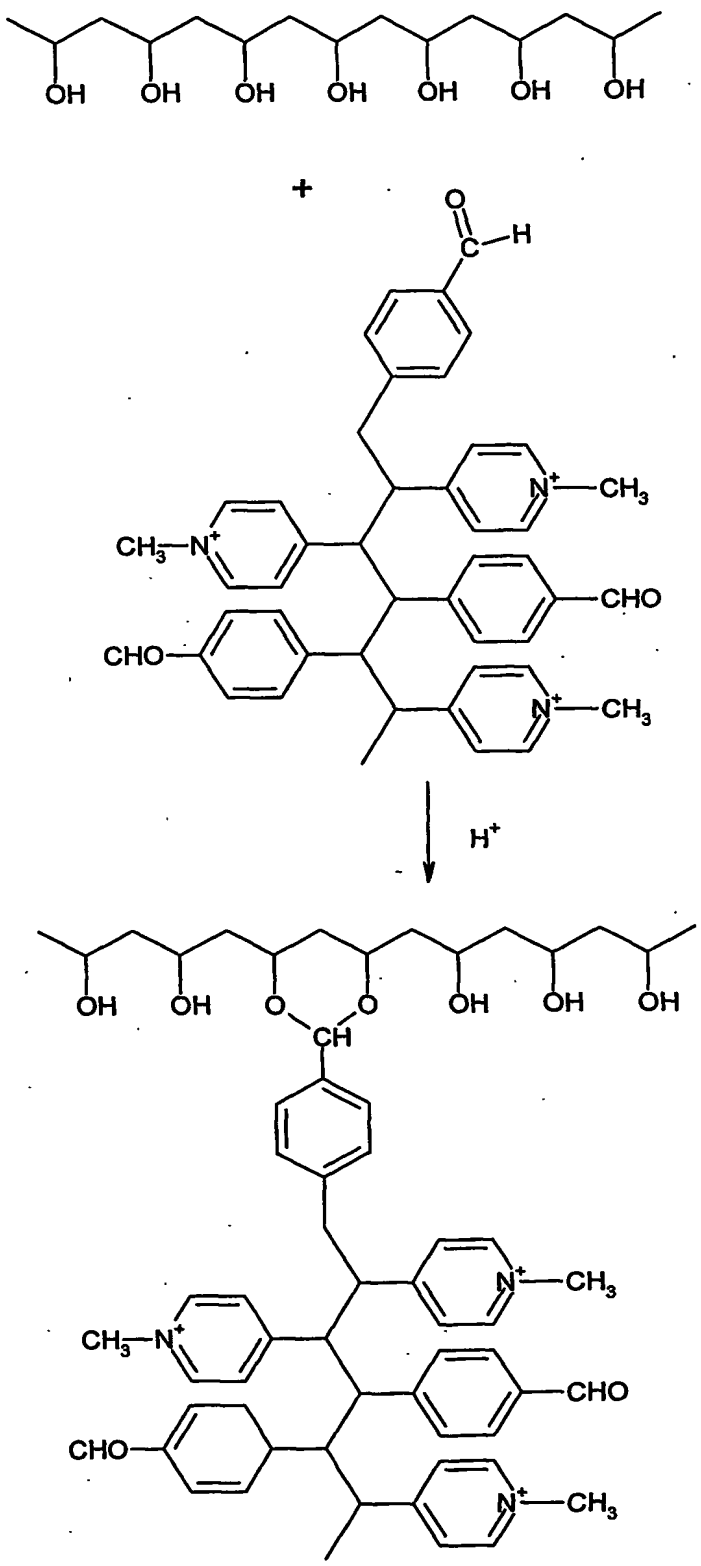
IX

Step (b)

A predetermined amount of 88% hydrolysed poly(vinylalcohol) of molecular weight 300,000 is dissolved in water by heating to 60°C for 6 hours. Then this is allowed to cool and a predetermined amount of the butylidene polymer of Step (a) is dissolved in the solution.

Step (c)

An acid catalyst is added to the blend of Step (b) suitably to produce a pH of about 2. The mixture is then left to polymerise whereby the butylidene polymer of Step (a) cross-links the polyvinylalcohol according to the scheme below.


OH
OH
OH
OH
OH
OH
OH
+
O
C
H
N+
CH3
CH3
N+
CHO
CHO
N+
CH3
H+
OH
OH
O
O
OH
OH
OH
CH
N+
CH3
CH3
N+
CHO
CHO
N+
CH3

The concentration of the acid affects the speed of the cross-linking reaction. As cross-linking occurs a hydrogel is formed which can be treated and/or manipulated as described herein.

The hydrogel prepared may include 80-90wt% of entrapped water.

Example 2 - General methodologies for preparing grating slides incorporating predetermined microtopography.

Oriented microtopographical cues are presented to cells as sets of grooves and ridges or channels embossed/cast into the surface of the gel using a master usually fabricated in fused silica or similar material. A desired microtopographic design is first drawn out as a lithographic resist mask either by photographic reduction or using a specific design package. A photoresist is patterned by exposure to UV through the lithographic mask followed by removal of exposed/non-exposed photoresist using a developing solution (either is possible depending on whether a positive or negative photoresist is used). This process may be used to fabricate features in the range of 10-100$\mu$m in linear dimension. To produce microtopographic features smaller than this (e.g. of 50nm to 10$\mu$m) electron beam resists, usually of PMMA, are exposed using a beam writer whereby the design is directly written into the surface of the resist. For both types of device a grating slide incorporating predetermined microtopography is created by reactive ion etching the surface of a fused silica blank with gases such as $C_2F_6$ or equivalent using the patterned resist as an etch mask for

sufficient time as is required to achieve a given etch depth.

Grating slides prepared may then be used to create microtopography on a hydrogel surface.

Example 3 - Preparation of cast hydrogel incorporating microtopography

A selected grating slide made as described in Example 2, was cleaned with 10% decon (a detergent) solution in an ultrasonic bath for 40 minutes, rinsed copiously in water, thoroughly dried and allowed to cool. The slide was then rinsed with 100% acetone and again allowed to dry. A petri dish was cleaned with heamosoal (a detergent), rinsed copiously in water, dried, and finally rinsed in 100% acetone, excess acetone being allowed to evaporate off. The slide was then placed in the petri dish, grating side up. A hydrogel formulation was prepared generally as described in Example 1 Steps(a)-(c), using 60g of a 10wt% solution of polyvinyl alcohol and 0.1 g of the polymer of Example 1, Step (c). The aqueous solution formed contains 10wt% polyvinyl alcohol and 0.5 wt% of the polymer of Example 1, step (c). The formulation in the presence of 1ml 20% HCl was mixed slowly, to reduce any air bubbles. The polymerising mixture was then poured into the petri dish and allowed to fully polymerise in a fume cupboard overnight. The dish was then placed into a vacuum oven overnight, at 50°C and -15mmHg pressure. After full polymerisation had occurred the hydrogel was peeled from the dish (and slide) and the grating formed in the hydrogel was cut out from the surrounding redundant

hydrogel. It is found, on microscopic examination, that the hydrogel is able to provide a very good reproduction of the grating.

Example 4 - Preparation of pulled hydrogel incorporating microtopography

A selected grating slide was prepared as described in Example 3 and, after drying, was placed on an inverted petri dish, grating side up. The hydrogel was prepared using 20g of a 10wt% solution of polyvinylalcohol and 0.1g of the polymer of Example 1, step (c). The aqueous solution formed contains 10wt% polyvinylalcohol and 0.5wt% of the polymer of Example 1, step (c). The aqueous solution was then mixed slowly with 0.2ml of 20% HCl, and then poured into a 5 cm petri dish to enable a hydrogel of a few mm thickness to be produced to enable it to be stretched. At a defined point in the polymerisation (when the hydrogel was not sticky enough to stick to a finger, but still quite 'wet') a corner of the hydrogel was taken in fingertips and stretched over the petri dish with grating. The hydrogel was left at room temperature for 1 hour and placed in an oven at 50°C for 45 minutes for full polymerisation to take place. After full polymerisation had occurred the hydrogel was carefully peeled from the dish (and slide) and the grating cut out from the redundant hydrogel.

Again, microscopic examination reveals that the hydrogel is able very precisely to reproduce the grating.

Example 5 - General procedures for derivatising hydrogels

Any of the hydrogels described herein (whether incorporating microtopography or otherwise) may be derivatised to improve their bio-compatibility and/or to increase adhesion to cells, and such derivatised hydrogels may be used in a wide range of applications. In preferred embodiments gels are dried prior to derivatisation. This may involve drying under vacuum at a temperature in the range 20 to 40°C for about 16 hours. Suitably, the gels are dried so that they contain less than 1wt% of water (measured e.g. by thermogravimetric analysis). It has been found that when dried gels are derivatised, the surface of the gel is predominantly derivatised (in preference to internal regions of the gel) from which a number of advantages may result. For example, bio-compatibility and/or the ability of the gels to adhere to cells will be concentrated at the surface of such derivatised gels, thereby restricting penetration into the body of the gel. Also, it is found that derivatisation may not significantly reduce the strength of the gels but may in some cases increase the strength. More particularly, derivatised dried gels may be rehydrated and the resultant hydrated derivatised gel may have comparable (or greater) strength compared to the non-derivatised version.

Hydrogels may be derivated using a range of methods. Firstly, a material, for example a bio-compatible material may be covalently bonded to the gel. In this regard, for the gel of Example 1 above, derivatisation may involve reactions involving hydroxyl, acetate or aldehyde groups

on the gel. A linking group may be attached to the gel via such groups and then a desired bio-compatible material may be covalently attached to the linking group. Examples of suitable linking groups include the following:

- the polymer of Examples 1, Step (a). The polymer has aldehyde groups which may be reacted with hydroxy groups of the cross-linked polymer of Example 1, Step (c).
- the monomer (SbQ) described in Example 1, Step (a). Again this monomer has aldehyde groups which can be reacted with hydroxy groups.
- the monomers described on page 3 line 8 to line 39 of GB 2030575B and polymers prepared therefrom as described according to WO98/12239.

Secondly, a bio-compatible material may be associated with the gel by formation of a charge transfer complex. More particularly, a complex may involve interaction with an $N^+$ moiety of a pyridinium moiety of the gel or a version of the gel derivatised with a pyridinum containing compound as described above. For example, a surfactant such as sodium lauryl sulphate may be attached to the gel using this methodology. In this case, the derivatised gel is less susceptible to subsequent swelling when hydrated and this may facilitate further reactions of the gel, for example of carbonyl groups thereof.

It will be appreciated that processes for derivatising the gel will be selected according to the nature of the bio-compatible material that it is desired to associate with the gel. In many embodiments, the bio-compatible material includes one or more amine groups (e.g. the material may

be a protein or amino acid), in which case the material may be covalently bonded to the gel by an amide bond. Examples of materials that may be bonded to the gel in this way and which may improve the gels compatibility with cells include all known extracellular matrix components, cytokines, growth factors, hormones and other intra- or extracellular signalling molecules. A specific example is fibronectin.

Example 6 - Derivatisation of hydrogel with fibronectin

Referring to figure 1, the hydrogel of Example 1, Step (c) (XI) having polyvinylalcohol moieties at its surface is treated with the butylidene polymer (XII) of Example 1, Step (b) in the presence of acetone and an acid thereby to produce the condensation product XIII. Product XIII is then treated with carbonyl diimidazole (XIV) in acetone to produce XV which is treated with aqueous fibronectin (XVI) to produce the fibronectin derivatised hydrogel XVII.

In more detail, 2g of the polymer of Example 1, step (c) is added to 100ml of acetone and the solution stirred for 2 hours. This produces a saturated solution of the polymer of Example 1, step (c) in acetone (excess un-dissolved polymer is seen at the bottom of the flask). 0.5ml of concentrated hydrochloric acid is added to the solution. The dry hydrogel film is then immersed in the acidified polymer/acetone solution for at least 4 hours but not more than 16 hours. (By way of example, a dry hydrogel film of dimensions approx. 10cm diameter by 0.5mm thick requires 50 ml of the solution). The film is then washed with acetone several times and dried at 25°C under vacuum for approximately 4 hours.

100ml of a 1%w/v solution of carbonyl diimidazole in acetone is prepared and the hydrogel film prepared above is immersed in the solution for 4 hours. The film is then removed and again washed with acetone several times and dried under vacuum at 25°C for 4 hours.

Exposure of the above film to an aqueous solution of fibronectin results in covalent attachment of the fibronectin to the surface. The film is then washed with sterile distilled water.

Example 7 - General procedure for producing micropatterned surface chemistry on the gel

Whilst the entire surface of the hydrogel may be derivatised as described in Examples 5 and 6, it is also possible to derivatise the gel in predetermined areas only thereby to produce desired surface chemistry in a predetermined pattern. A first method of achieving this involves the use of an elastomer stamp which has desired microtopography cast (or otherwise formed) into its surface. The elastomer is cured and soaked in a solution of an amine group containing compound (e.g. a protein such as fibronectin) for a time (about 20 minutes) for it to absorb sufficient of the protein for the application. The stamp is briefly exposed to a nitrogen stream to dry it. It is then placed contact side downwards on a hydrogel surface which has been derivatised with appropriate functionality so that it can react with the amine groups of the amine group containing compound (e.g. the stamp may be used to deliver fibronectin (XVI) to a gel derivatised as per compound XV of figure 1). After sufficient contact

time (e.g. 1 minute) the stamp is withdrawn, leaving covalently bonded amine compound in predetermined positions on the gel. Thereafter, optionally, the non-derivatised areas may be treated with an alternative amine-group containing compound. The latter compound may be selected on the basis of the combinatorial effects of the two amine compounds to bind cells etc or it may act as a blocker for cell attachment. Examples of blockers are albumin and casein.

A second method of providing micropatterned surface chemistry involves the use of a stamp as described above except that in this case the stamp is planar. The stamp, loaded with amine containing compound, is moved by an operator to selected positions and contacted with the gel surface to define desired surface chemistry at the selected positions.

Use of materials prepared

A gel, for example in the form of a film, incorporating microtopography and/or micropatterned surface chemistry may be formed in a suitable size to act as a dressing for a wound. The dressing is then applied engineered side down onto the wound itself. The wound may optionally be pre-treated by dermabrasion (or the like) to achieve appropriate viable cell-hydrogel contact. Studies on human skin cells and on model wounds have shown that the dressing can facilitate wound closure and/or the healing process.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to

this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.